In the Claims

Cancel Claims 49 and 51.

REMARKS

Claims 49 and 51 have been canceled.

Comments Regarding Examiner's Discussion of Declaration of Jonathan S. Stamler, M.D. Under 37 C.F.R. § 1.132; Comments Apply to Rejections in Which WO 93/09806 Has Been Cited

The Examiner concludes that the reagent used in experiments to attempt the synthesis of SNO-hemoglobin was SNOAc. The Examiner bases this conclusion on page 58, lines 17-25 of Example 19 in WO 93/09806 and on Figures 1 and 2 of 08/559,172, now abandoned, which the Examiner observes are the same spectra as Figures 28 and 29 of WO 93/09806. It should be noted that in the interview conducted on August 5, 1999 at the United States Patent and Trademark Office, Dr. Stamler stated that the Examples in WO 93/09806 and 08/559,172 were attempts to describe the same experiments, and that both Examples were inaccurate in similar ways. Among the inaccuracies was the identification of the reagent in the first paragraph of the Examples as SNOAc, when, according to Dr. Stamler, the reagent used in that experiment was acidified nitrite. See Exhibits A and B accompanying the Declaration of Jonathan S. Stamler, M.D. Under 37 C.F.R. § 1.132 mailed to the United States Patent and Trademark Office on September 2, 1999. Thus, it is not appropriate to cite 08/559,172 as proof that SNOAc is the correct reagent. The Examiner should also note that the method to allegedly produce *S*-nitrosohemoglobin appearing in Example 19 of WO 93/09806 and Example 1 of 08/559,172 was not published in a scientific journal, but the method described in the subject patent application was published in the refereed journal *Nature* in March, 1996, after the filing date of the priority patent application [Jia, L. *et al., Nature 380*:221-226 (21 March 1996)].

The Examiner sets a new standard for Applicants to meet in the Examiner's determination of whether a reference sets forth an enabling description of an invention, with the requirement that Applicants prove the absence of SNO-hemoglobin. This standard appears nowhere in the rules or in patent law. This standard is impossible to meet; it is impossible to prove the complete absence of anything.

The standard for determining whether a prior art reference is enabling has been set forth by the Federal Circuit in *In re Donahue* 226 USPQ 619, 621 (Fed. Cir. 1985).

> It is well settled that prior art under 35 U.S.C. § 102(b) must sufficiently describe the claimed invention to have placed the public in possession of it. *In re Sasse*, 629 F.2d 675, 681, 207 USPQ 107, 111 (CCPA 1980); *In re Samour*, 571 F.2d at 562, 197 USPQ at 4; see also *Reading & Bates Construction Co. v. Baker Energy Resources Corp.*, 748 F.2d 64, 651-52, 223 USPQ 1168, 1173 (Fed. Cir. 1984). Such possession is effected if one of ordinary skill in the art could have combined the publication's description of the invention with his own knowledge to make the claimed invention. See *In re Grice*, 301 F.2d at 939, 133 USPQ at 373-74. Accordingly, even if the claimed invention is disclosed in a printed publication, that disclosure will not suffice as prior art if it was not enabling. *In re Borst*, 345 F.2d. 851, 855, 45 USPQ 554, 557 (CCPA 1965), *cert. denied* 382 U.S. 973, 148 USPQ 771 (1966).

"When the reference relied upon expressly anticipates or makes obvious all of the elements of the claimed invention, the reference is presumed to be operable." MPEP 2121. *In re Sasse*, 629 F.2d 675, 207 USPQ 107 (CCPA 1980). While Applicants do not believe that WO 93/09806 "anticipates or makes obvious all of the elements of the claimed invention, Applicants' task as to operability in the references after a 35 U.S.C. § 102 or § 103 rejection has been made is to rebut, by a declaration, the *presumption* of operability by a preponderance of the evidence. Applicants have met this burden.

The Examiner goes on to say in his discussion of the Declaration:

> Applicant's own specification demonstrates that reacting a low molecular weight *S*-nitrosothiol such as SNOAc in equimolar amounts with hemoglobin (e.g. deoxy or oxy) would be expected to generate SNO-hemoglobin (e.g. see present specification at pages 46-48 and Figures 1a-1d).
> It is also noted that use of extrinsic evidence by the Examiner to demonstrate inherency is permitted (e.g. see MPEP 2131.01(d)), including the use of applicant's own specification (e.g. examples). See *Ex parte Novitski*, 26 USPQ2d 1389 (B.P.A.I., 1993).

It is assumed the Examiner meant to say pages 57-59 discussing the results shown in Figures 1a-1d, instead of pages 46-48. It is not clear to Applicants how inherency is being used. The Examiner has not recited specific claims, or a specific property or element of the invention that is allegedly inherent, nor has he provided reasoning to support a conclusion of inherency in that context. Rather, the Examiner is attempting to use the theory of inherency in his assessment of the persuasiveness of the Declaration of Jonathan S. Stamler, M.D. Under 37 C.F.R. § 1.132 on the question of whether WO 93/09806 presents an enabling description of a synthesis of SNO-

hemoglobin. The theory of inherency does not apply in this assessment, only in making a rejection under 35 U.S.C. § 102 or § 103. In any case, a conclusion -- for example, that SNOAc and hemoglobin *would be expected* to generate SNO-hemoglobin -- cannot be inherent. A result of an untested method cannot be inherent; only what is ***necessarily*** true can be concluded to be inherent.

It is appropriate to consider a reference for whether it contains an enabling description only by considering the reference and other knowledge available to one of ordinary skill in the art at the time of Applicants' priority date. Applicants' specification was not available to one of ordinary skill in the art at the priority date.

> A reference contains an "enabling disclosure" if the public was in possession of the claimed invention before the date of invention. "Such possession is effected if one of ordinary skill in the art could have combined the publication's description of the invention with his [or her] own knowledge to make the claimed invention." *In re Donohue*, 766 F.2d 531, 226 USPQ 619 (Fed. Cir. 1985).

The Examiner states, "The Declarant's attempt to reproduce the Reference Example 19 method was not found persuasive since it is unclear as to whether applicant is showing the absence of SNO-hemoglobin or the inability of the utilized assay to detect the presence of SNO-hemoglobin." Exhibits E1-E3 accompanying the Declaration of Jonathan S. Stamler, M.D. include a standard "curve" which demonstrates that the assay can be used to detect low amounts of *S*-nitrosothiol. Dr. Stamler has indicated in the Declaration (see statement 6 of the Declaration) that this improved assay is being used now in his laboratory to assay for SNO-hemoglobin and that the unimproved assay that was used several years earlier in an attempt to detect SNO-hemoglobin in Example 19 of WO 93/09806 could not have detected SNO-hemoglobin even if it had been made. The assay used to produce Exhibits E1-E3 with the Declaration was the most sensitive method known; no method to assay for SNO-hemoglobin was available before the time of Applicants' priority document. Moreover, as Dr. Stamler further stated in the Declaration, an essential step to separate the product nitrosothiol from the reagent nitrosothiol was never performed. Thus, any assay performed in this manner in an attempt to detect nitrosothiol would give a false positive result. From the "analysis" presented in Example 19 of WO 93/09806, one of ordinary skill in the art could not conclude that SNO-hemoglobin had been made. Further, there is no guidance found in the prior art on how to produce other *S*-nitrosylated proteins that have been found to retain their physiological function.

The Examiner refers also to Example 1 and Figures 1 and 2 in 08/559,172 (now abandoned), as a document "which confirms the presence of a 'composition which comprises SNO-hemoglobin' within the scope of the presently claimed invention." Again the Examiner refers to 08/559,172 as showing "the formation of *S*-nitrosyl hemoglobin at this pH [6.9] in addition to higher pH optimums." The Declarant explained in the interview that 08/559,172 and WO 93/09806 described the same experiments and contained similar errors in the description and conclusions of those experiments. Thus, the Declaration should serve to rebut statements in 08/559,172 as well as in WO 93/09806.

The Examiner states that one would be motivated to optimize reaction parameters such as pH and reagent concentration as a matter of course. One of ordinary skill in the art, concluding from Example 19 that synthesis of SNO-hemoglobin was not shown, would have no motivation to take up any experiments to produce SNO-hemoglobin or any guidance about what to try. It was not obvious to increase the concentration of the reagent, as it was thought that an increase in the NO· generated would cause too much oxidation of the hemoglobin. Dr. Stamler stated in the interview that it had been thought, before the time of the priority application, that an acidic pH was critical, and that neutral and basic pHs were to be avoided. It was thought that SNOAc and other nitrosothiols would break down and release NO· at alkaline pH, which would cause the oxidation of hemoglobin. Therefore, it was not obvious that a higher pH should be used to *S*-nitrosylate hemoglobin. It was also pointed out in the interview that, according to the prior art, higher concentrations of NO-donor were to be avoided because NO donors were known to oxidize hemoglobin. It was first appreciated by Applicants that the rate of *S*-nitrosylation of hemoglobin and the channeling of NO from hemes to thiols to form SNO-hemoglobin are dependent on the conformational state of hemoglobin (T versus R) associated with an alkaline pH. See page 59, lines 5 to 20 and page 97, lines 3-17, for instance.

The Examiner states:

> . . . applicant's claims are not restricted only to the use of an *S*-nitrosylated hemoglobin compound but are generically broader to include a mixture of *S*-nitrosylated hemoglobin and other hemoglobin derivatives to which the Stamler reference is enabled by its disclosure and its examples taken separately or in view of obvious modifications thereof (e.g. optimization of reactions and experimental conditions).

Applicants' claims are as broad as the use of nitrosated or nitrated hemoglobin. The Stamler reference discusses the theoretical use of compositions comprising *S*-nitrosohemoglobin or other

S-nitrosoproteins in methods of therapy to alleviate various conditions by delivery of NO to tissues. However, WO 93/09806 does not describe a successful synthesis of any species of *S*-nitrosohemoglobin, and reactions that are not successful cannot be optimized. The only other species of nitrosated or nitrated hemoglobin known at the time of Applicants' invention was NO-Hb (nitrosylhemoglobin, which has NO bound to the heme Fe and does not itself act as a donor of NO). There had been no suggestion in WO 93/09806 or elsewhere that NO-Hb can be useful in a method of therapy to deliver NO to tissues. Applicants were the first to show that NO-Hb can be converted to *S*-nitrosohemoglobin (see Example 19, pages 87, lines 1-11).

The Examiner states, "Additionally, the Examiner cites support for the proposition that it was well within the ordinary skill of the art to synthesize various nitrosylated hemoglobin compositions." Applicants do not see any cited support for this proposition beyond the Examiner's assertion that WO 93/09806 describes the synthesis of SNO-hemoglobin.

Rejection of Claims 15, 16, 17 and 59 Under 35 U.S.C. 103(a)

Claims 15, 16, 17 and 59 have been rejected under 35 U.S.C. 103(a), as they are said to be unpatentable over Stamler *et al.* (WO 93/09806).

Stamler *et al.* (WO 93/09806) disclose *S*-nitroso-proteins, in particular, *S*-nitroso-tPA, *S*-nitroso-BSA, *S*-nitroso-cathepsin B, *S*-nitroso-lipoprotein and *S*-nitroso-immunoglobulin, and methods for producing the same, using NO or $NaNO_2$ as the reagent, under acidic conditions. They also report a method which they claim results in the synthesis of *S*-nitroso-hemoglobin. However, this compound was not produced by any method reported in WO 93/09806, as attested to in the Declaration of Jonathan S. Stamler, M.D. Under 37 C.F.R. § 1.132 submitted to the United States Patent and Trademark Office on September 2, 1999. Methods used to synthesize other *S*-nitroso-proteins, which might have been expected to nitrosate or polynitrosate hemoglobin, dissociated hemoglobin into its subunits, oxidized the heme Fe and rendered the product useless for carrying oxygen. Methods described in the specification that result in the synthesis of nitrosated hemoglobins are substantially different from both the acidified nitrite method described in WO 93/09806, which was unsuccessful on hemoglobin, and the methods using NO in solution to nitrosylate other proteins.

The existence of all but one species of nitrosated or nitrated hemoglobin -- nitrosylhemoglobin (which has the NO bound to the heme iron and is not itself a donor of NO) --

was unknown before the invention. No person of ordinary skill in the art would be able to conclude from WO 93/09806 that any other species of nitrosated or nitrated hemoglobin had been synthesized. Example 19 of WO 93/09806 presents a number of errors in reporting the procedures that were followed, in the logic applied to the results, and in the conclusions that were drawn from those results. These errors are described in the paragraphs that follow.

One matter is the missing reagent on line 5 of page 58 of WO 93/09806. Although the context of Example 19 might suggest that the reagent is SNOAc (*S*-nitroso-*N*-acetylcysteine), the laboratory notebook records of Dr. Stamler show that the reagent used for the experiment, the results of which are shown in Figure 28, was actually acidified nitrite, the only reagent which had been known, by the prior art, to yield other SNO-proteins. See Exhibits A and B with the Declaration of Jonathan S. Stamler, M.D. Under 37 C.F.R. § 1.132 filed with the United States Patent and Trademark Office on September 2, 1999.

If we assume that the reagent was SNOAc, then the Saville assay as described in Example 19 of WO 93/09806 could not have yielded interpretable results. Example 19 includes no report of a separation step to separate the reagent, a low molecular weight *S*-nitrosothiol, from the product, which the Examiner presumes to be SNO-hemoglobin, a high molecular weight protein. Thus, the assay would give the misleading result, with SNOAc, and in all such cases where a low molecular weight *S*-nitrosothiol is used as a reagent, that an *S*-nitrosothiol product was formed, as the assay would detect the reagent. In fact, no separation step was performed, as Dr. Stamler has stated in the Declaration. See the second paragraph of statement 6 of the Declaration.

A minor matter is the number given as the absorption maximum of 450 nm as reported on line 14, for Figure 28. It can be seen from Figure 28 that this maximum is 540; one might assume the units are nanometers for the x-axis; no units are given for the y-axis. However, a more important matter is that it is not reported what solution produced this spectrum. It is not recognizable as a spectrum of hemoglobin. SNO-hemoglobin has no characteristic spectrum that is distinguishable from any other species of hemoglobin, as one of skill in the art would know. If the spectrum in Figure 28 is intended to be that of the azo dye which is generated from nitrosothiol in the Saville assay, it is not informative, as the Saville assay requires measurements made both in the presence and absence of mercuric ions, to assay for *S*-nitrosothiol. Figure 28 as shown in WO 93/09806 is a measure of nitrite, not *S*-nitrosothiol.

The sentence at lines 15-16 of page 58 of WO 93/09806 makes no sense, as the "*S*-nitrosothiol bond formation" being referred to in this sentence is supposed to be in SNO-hemoglobin, and could not be "demonstrated" in any way by "using NO^+ equivalents in the form of SNOAC."

The conclusions of the second paragraph on page 58 of WO 93/09806 are incorrect. It is impossible to tell from Figure 29 which line of the spectrum in the region of approximately 540-580 nm can be attributed to a particular synthesis procedure, as the lines of the five different spectra in the figure are not identified, and the lines of the spectra overlap. In any case, that region of the hemoglobin spectrum is difficult to interpret in general, as several species of hemoglobin absorb in that range. Note, for example, that the spectrum of NO(FeII)hemoglobin in the region of 540-580 nm resembles that of oxyhemoglobin. See Exhibit Z. Thus, the relative contributions of each of these species to the various spectra cannot be determined from the figure. What is clear, however, to one of skill in the art is that Figure 29 shows multiple hemoglobin derivatives in which the redox metal sites are different. The conclusion of one of skill in the art is the opposite of the conclusion stated in WO 93/09806 that "the UV spectrum of hemoglobin incubated with SNOAc shows no reaction at the redox metal (iron-binding site) of hemoglobin, over 15 minutes."

Figure 30, referred to on page 58, lines 19-25 of WO 93/09806, is said to be the spectrum of nitrosyl-hemoglobin. However, Figure 30 is not helpful for purposes of comparison with any of the other spectra, being plotted as a separate graph.

Lines 23-25 of page 58 draw the conclusion, "The fact that the *S*-nitrosothiol did not react with the redox metal site of hemoglobin, but with its thiol group instead, indicates that the reactive NO species donated by the *S*-nitrosothiol is nitrosonium or nitroxyl." No such conclusion can be drawn. On the contrary, one skilled in the art would conclude that there was a reaction with the redox metal site of hemoglobin. There is no evidence, from any of the assays or spectra examined, that *S*-nitrosothiol groups are present on a hemoglobin product, and no conclusion can be made about any reactive NO species from any of the experiments that might be described in Example 19.

Lines 26-27 of page 58 state, without any evidence, "*S*-nitrosylation of hemoglobin does not result in the formation of methemoglobin and consequent impairment in hemoglobin-oxygen

binding." On the contrary, methemoglobin is definitely formed by the processes one might assume to be described in Example 19, as explained below.

The sentence on page 58, line 28 to page 59, line 1 describes "a leftward shift in the hemoglobin-oxygen association curve." This leftward shift was merely a result of the presence of methemoglobin. Experiments to produce a hemoglobin-oxygen association curve are done in a tonometer of various oxygen partial pressures. The presence of substantial amounts of methemoglobin (λ_{max} = 405 nm) are well known to cause this shift. This has been misinterpreted by the drafters of WO 93/09806 as an increase in oxygen binding. The first sentence on page 59 continues, "Thus, the reaction between *S*-nitrosothiols and hemoglobin not only eliminates the inhibition of oxygen binding which occurs from the reaction with uncharged NO and generation of methemoglobin, but it actually increases oxygen binding." In the absence of any evidence of a reaction between *S*-nitrosothiols and the thiols of hemoglobin, the skilled person could not conclude what effect SNO-hemoglobin might have on oxygen binding.

The interpretation of hemoglobin spectra is not straightforward because of the many hemoglobin species with similar spectra. See, for example, the spectra of NO-hemoglobin in the Soret region and in the visible range (Exhibits Y and Z, respectively, which were produced in the laboratory of Dr. Stamler). The conclusion of WO 93/09806 that "the reaction between *S*-nitrosothiols and hemoglobin . . . actually increases oxygen binding" is unsupported. There are several alternative interpretations of the spectra of Figure 29 that are more plausible to one of skill in the art; all of them include reactions taking place at the metal redox center, contrary to the unsupported statements of WO 93/09806. None of the spectra of Figure 29 can be identified as being attributable to any one species of hemoglobin. To get an accurate measurement of the relative contributions of oxy-, NO(FeII)- or met-hemoglobin species among a mixture of hemoglobin species, it would be necessary to determine the amounts of the species oxy-, NO(FeII)-, and met-hemoglobin, using methods such as those reported by Gow, A.J. *et al., Proc. Natl. Acad. Sci. USA 96*:9027-9032, 1999 (Exhibit X). This was not done in this case, and these methods were not known at the time of the experiments of WO 93/09806. However, it is clear that the products from all of the procedures 1-4 (as these procedures are summarized on Exhibit C with the Declaration of Dr. Stamler) include significant amounts of one or more species of hemoglobin that cause a leftward shift in the peaks of the Soret region of the spectrum. It is difficult to determine the exact peaks, because the measurements were not made, or not given in

Example 19. However, it is clear that the leftward shift from the "middle spectra" to those in spectra 3 and 4 is at least 5 nm, and that, overall, the peaks in the Soret region cover a range of at least 10 nm. The explanation for the leftwards shift is the presence of significant methemoglobin, as the maximum absorbance for oxyhemoglobin is about 415 nm, the maximum for NO-hemoglobin (nitrosyl-hemoglobin, having NO bound to FeII of the heme) is about 417 nm, and the maximum for methemoglobin is about 405 nm. The spectrum of SNO-hemoglobin has no feature to distinguish it from the spectrum of oxyhemoglobin. (Moreover, the maximum for deoxyhemoglobin is 430 nm. At least one of the spectra would appear to have a partial deoxy component that would greatly confound the interpretation.) For procedures 1 and 2, the product has a maximum in the Soret region at 417 nm, which may indicate the presence of NO(FeII)hemoglobin, and for procedures 3 and 4, in which a higher concentration of SNOAc was used, there has been a leftwards shift, indicating that a major product is methemoglobin. The spectra cannot tell one of skill in the art anything about whether a reaction occurred at the thiols of the cysteine residues of hemoglobin; only that reactions are definitely occurring at the redox metal center.

An alternative explanation for the leftward shift in the hemoglobin absorbance spectrum is that the methemoglobin present promotes the R (high affinity) structure. That is, if the hemes of one or more subunits of the hemoglobin tetramer are oxidized, the remaining subunits bind oxygen more readily than they would if the subunits were in the deoxy (T structure) state.

In any case, the experiment of procedures 1 and 2 of Exhibit C has been repeated, and the result was that, by an improved Saville assay modified from that known at the time of the publication of WO 93/09806, no SNO-hemoglobin was produced. See statement 6 of the Declaration of Dr. Stamler, and accompanying Exhibits E1-E3. Figure 29 is, therefore, uninterpretable, but the conclusion of one of ordinary skill in the art could not be, seeing the maximum absorbance leftward shifted as it is, that (page 58, lines 17-19) "the UV spectrum of hemoglobin incubated with SNOAC shows no reaction at the redox metal (iron-binding site) of hemoglobin, over 15 minutes."

As can be seen from consideration of the above several points, the disclosure of WO 93/09806 contains statements that are in error, as well as statements and figures that are inconsistent and uninterpretable, such that the person of ordinary skill in the art would not be

able to learn from it how to make or use SNO-hemoglobin or any other form of nitrosated or nitrated hemoglobin.

The Examiner states:

> . . . it would have been prima facie obvious to one of ordinary skill in the art at the time of applicant's invention to utilize nitrosylated hemoglobin for purposes of inhibiting platelet activation, preventing thrombus formation or for treating platelet activation or adherence disorders including cardiovascular disorders (e.g. infarction, embolism etc.) by administration of nitrosylated hemoglobin to a patient in need thereof as described in the Stamler reference.

It may have been desirable to use a donor of NO in a method of therapy to treat medical disorders arising from platelet activation. However, at the time of the invention, no form of nitrosylated hemoglobin was known to be a donor of NO. Unmodified hemoglobin was known in the scientific literature to be a *scavenger* of NO, blocking NO biological activity (see Wennmalm Å., *et al., Br. J. Pharmacol. 106*:507-508, 1992; reference AS5). Hemoglobin is known to activate platelets (see, for example, abstract of Olsen, S.G. *et al., Circulation 93*:327-332, 1996; cited as reference AY). Before the time of the invention, it was not known that SNO-oxyhemoglobin, unlike other forms of hemoglobin, does not produce the physiological effects of an NO donor and does not inhibit platelets. (See, for example the written description at page 79, line 8 to page 80, line 10, and compare Figure 7B with Figures 7A and 7C.)

Before the invention, it was known that low molecular weight nitrosothiols were desirable as vasodilators and platelet inhibitors. Low molecular weight thiols were not reported to have any biological activity on their own. Hemoglobins, with the heme Fe in an unoxidized state, were known to be carriers of oxygen. Before the invention, it was not known that any form of nitrosylated hemoglobin could function as a carrier and donor of NO (SNO-hemoglobin was unknown), and that NO could be transferred from hemoglobin to thiol, and from nitrosothiol to hemoglobin. Therefore, there was no motivation to combine a low molecular weight thiol or nitrosothiol with hemoglobin or nitrosated hemoglobin, as in Claim 16.

Rejection of Claims 15 and 16 Under 35 U.S.C. § 103(a)

Claims 15 and 16 have been rejected under 35 U.S.C. § 103(a), as they are said to be obvious over Stamler *et al.*, WO 93/09806 and Kaesemeyer, US 5,543,430.

The Examiner states:

> Stamler *et al.* generally teach the use of NO donor nitrosylated protein compounds (e.g., the addition of an NO group to an SH, oxygen, carbon or nitrogen; see page 14, lines 7-12), including nitrosylated hemoglobin formed by conventional means (e.g. see Stamler page 1-5), for use in relaxing smooth muscle, inhibiting platelet aggregation (e.g. preventing thrombus formation), promoting vasodilation and for treating/preventing cardiovascular disorders (e.g. see page 19, lines 22-25; Stamler claims 18, 20, 36, 37, 41-42, 44, 45; etc.). Cardiovascular disorders include those within the scope of the presently claimed invention (e.g. myocardial infarction, pulmonary embolism, etc. (e.g., see page 18, lines 5-11).

The teachings of Stamler *et al.* (WO 93/09806) have been described above. As explained above regarding WO 93/09806, methods of making several nitrosylated proteins are taught. However, no successful synthesis of *S*-nitrosylated, *O*-nitrosylated, *C*-nitrosylated, or *N*-nitrosylated hemoglobin is taught. It might have been desired to use a donor of NO in a method of therapy to treat a disorder arising from platelet activation. However, at the time of the invention, no modified form of hemoglobin was available to try to produce these effects. NO-Hb (nitrosylhemoglobin) was not known to be useful as a donor of NO, as it was not known at that time that it could be converted, under physiological conditions, to SNO-hemoglobin, which is a donor of NO.

The Stamler *et al.* published patent application WO 93/09806 does not and cannot teach the use of nitrosylated *hemoglobin* as an NO donor for use in relaxing smooth muscle, for inhibiting platelet aggregation, or for anything else. As can be concluded from the Declaration of Jonathan S. Stamler, M.D. Under 37 C.F.R. § 1.132, filed on September 2, 1999, no form of nitrosated, nitrated, or nitrosylated hemoglobin is enabled by the disclosure of WO 93/09806. The description of a method to produce *S*-nitrosohemoglobin appearing in WO 93/09806 was followed in an attempt to reproduce the method, as explained in the Declaration, and did not produce *S*-nitrosohemoglobin. WO 93/09806 does not and cannot disclose or predict the finding that one form of SNO-hemoglobin, SNO-oxyhemoglobin, does not act as a donor of NO and does not cause the physiological effects of NO, unlike other *S*-nitrosothiols in the prior art. See Figure 20A and Example 22 of the written description, especially page 90, lines 4-20.

Kaesemeyer (U.S. Patent No. 5,543,430) describes a method for treating cardiovascular diseases using L-arginine and a vasodilator, a compound "which when administered to a subject is converted biologically to nitric oxide (NO) which is a pharmacologically active metabolite." See column 1, lines 21-24. *S*-nitrosothiols are mentioned as being vasodilators. See column 1, line 49.

Nitrates are also mentioned by Kaesemeyer as a source for the production of NO. See column 6, lines 19-23, and lines 44-47. Kaesemeyer does not mention any form of hemoglobin, or any nitrosoprotein. One of ordinary skill in the art would find no reason in Kaesemeyer or elsewhere to turn to any form of hemoglobin as a possible donor of nitric oxide. It was thought at the time of the filing of the Kaesemeyer application (October 5, 1994), that even if hemoglobin could be nitrated or nitrosated, it would *block* nitric oxide activity by acting as a scavenger of nitric oxide. It was also known that the administration of the combination of hemoglobin and the nitrate nitroglycerin to patients would result in methemoglobinemia (see, for example, abstract and first paragraph in Kaplan, K.J. *et al., The American Journal of Cardiology 55*:181-183, 1985; copy provided as Exhibit V).

From the combination of cited references, one of ordinary skill in the art might seek an *S*-nitrosothiol of some kind to be used as an inhibitor of platelet activation, and might turn to an *S*-nitrosoprotein as a candidate vasodilator. However, from other art known at the time of filing the priority application (see, for example, Greenburg, A.G., and H.W. Kim, *Art. Cells, Blood Subs. and Immob. Biotech., 23(3)*:271-276, 1995; cited as reference AZ3), one would know that hemoglobin (any form that is not oxidized at the heme Fe) was thought to act as a scavenger of nitric oxide. See, for instance, page 273, lines 12-24 of Greenburg *et al.* This would discourage investigations into the use of any form of hemoglobin as an inhibitor of platelet activation. How could hemoglobin act as a donor of NO and produce a biological effect, if it is also an effective scavenger of NO, ready to bind free NO at the heme Fe? Also, why use an NO donor if NO is known to react with oxyhemoglobin to produce methemoglobin (see page 272, lines 40-41 of Greenburg and Kim, reference AZ3), which can have adverse physiological effects?

Presented with a description of a method to produce SNO-hemoglobin in WO 93/09806, one of skill in the art would be unable to follow it to produce SNO-hemoglobin.

The Examiner states:

> The Stamler et al. generic teaching of using NO donor nitrosylated protein compounds, including nitrosylated hemoglobins and methemoglobins, and a species of specifically nitrosylated hemoglobin (e.g. *S*-nitrosylated) would motivate one of ordinary skill in the art to utilize other nitrosylated hemoglobins which would be deemed to be functionally equivalent as NO donating compounds.

WO 93/09806 does not contain a generic teaching of using "nitrosylated hemoglobins and methemoglobins." The only species hypothesized, but not shown by any data, to be a donor of

NO is "*S*-nitrosohemoglobin." There is no teaching in WO 93/09806 or in Kaesemeyer, or in the combination of these references, of what other species of hemoglobin might function as NO donating compounds.

The Examiner continues:

> Thus, the selection of a species of NO donating hemoglobin is a matter of choice to one of ordinary skill in the art since the Stamler reference teaches the functionally equivalent use of nitrosylated proteins, including hemoglobins, as well as individual nitrosylated hemoglobin species including thionitrosylated hemoglobin.

The Stamler reference discusses only one form of hemoglobin as if it had been made and could be used as a donor of NO. That is "*S*-nitrosohemoglobin." No other form of hemoglobin is hypothesized in WO 93/09806 as being a donor of NO. Contrary to what might have been predicted from the *S*-nitrosoproteins successfully produced as described in WO 93/09806, one species of SNO-hemoglobin, SNO-oxyhemoglobin, does not act as a vasodilator (See, for example, Figure 4A, page 68, lines 3-14, Figure 20A and Example 22, especially page 90, lines 4-20 in the written description) or as an inhibitor of platelet activation. (See, for example in the written description, page 79, line 8 to page 80, line 10, and compare Figure 7B with Figures 7A and 7C.)

The Examiner states:

> In this regard, the use of oxidized forms of NO (e.g. nitrates/nitrites) as functionally equivalent NO donors is conventionally known in the art (e.g. see Kaesemeyer at col. 6).
> Accordingly, it would have been obvious to one of ordinary skill in the art at the time of applicant's invention to substitute nitrated hemoglobins for nitrosohemoglobins in the methods as disclosed by Stamler with a reasonable expectation of success due to functional equivalency of nitrites/nitrates as NO donating compounds.

What Kaesemeyer says regarding nitrates appears in column 6, lines 44-47: "Nitrates such as isosorbide dinitrate, and isoasorbide 5' mononitrate also can be used to produce NO since they are simply commercially available intermediates to the known L-arginine independent pathway." Nitrates are also mentioned as a source for the production of NO. See column 6, lines 19-23, and lines 44-47. Kaesemeyer does not mention any form of hemoglobin, or any nitrosoprotein. Kaesemeyer does not say that nitrate-modified hemoglobin, nitrate-modified proteins in general, or even inorganic nitrates are functionally equivalent NO donors. It was

known from the prior art that nitrates oxidize hemoglobin to form methemoglobin both *in vitro* and in patients receiving nitrates. See Kaplan *et al.* (Exhibit V), for example the abstract and first paragraph. Furthermore, it was thought, before the time of the priority application, that hemoglobin would *block* nitric oxide activity by acting as a scavenger of nitric oxide, with the heme iron becoming oxidized so that it cannot carry oxygen.

Claims 15 and 16 do not recite anything about nitrites. Kaesemeyer teaches nothing about nitrites. WO 93/09806 teaches the use of acidified nitrite to produce some SNO-proteins other than SNO-hemoglobin, but does not teach or suggest "functional equivalency of nitrites/nitrates as NO donating compounds" or that nitrites can be NO donating compounds at all. From the prior art, one of ordinary skill would know that nitrite ions cause the oxidation of hemoglobin to methemoglobin, and can cause methemoglobinemia. See, for example, the abstract in Doyle, M.P. *et al., Journal of Free Radicals in Biology and Medicine 1*:145-154, 1985; copy provided as Exhibit W.

The Stamler *et al.* published patent application WO 93/09806 does not and cannot teach the use of NO donor nitrosylated *hemoglobin* for use in relaxing smooth muscle, for inhibiting platelet aggregation, or for anything else. As can be concluded from the Declaration of Jonathan S. Stamler, M.D. Under 37 C.F.R. § 1.132, filed on September 2, 1999, no form of nitrosated, nitrated, or nitrosylated hemoglobin is enabled by the disclosure of WO 93/09806. The description of a method to produce *S*-nitrosohemoglobin appearing in WO 93/09806 was followed in an attempt to reproduce the method, as explained in the Declaration, and did not produce *S*-nitrosohemoglobin.

From the combination of cited references, one of ordinary skill in the art might seek an *S*-nitrosothiol of some kind to be used as an inhibitor of platelet activation, and might turn to an *S*-nitrosoprotein or a nitrated protein as a candidate vasodilator. However, from other art known at the time of filing the priority application (see, for example, page 273, lines 12-24 of Greenburg, A.G., and H.W. Kim, *Art. Cells, Blood Subs. and Immob. Biotech., 23(3)*:271-276, 1995; cited as reference AZ3), one would know that hemoglobin was thought to act as a scavenger of nitric oxide and that NO donors can oxidize hemoglobin (see Kaplan *et al.*, Exhibit V). This would discourage investigations into the use of any form of nitrosylated or nitrated hemoglobin as a vasodilator or as an inhibitor of platelet activation. Presented with a description of a method to

produce SNO-hemoglobin in WO 93/09806, one of skill in the art would be unable to follow it to produce SNO-hemoglobin, and Kaesemeyer cannot make up for this deficiency.

Rejection of Claims 15-17 and 49-59 Under 35 U.S.C. § 103(a)

Claims 15-17 and 49-59 have been rejected under 35 U.S.C. § 103(a), as "obvious over Stamler et al., WO 93/09806 (5/93) alone and if necessary further in view of the specification admission as to prior art on pages 37-39, Kaesemeyer, U.S. Pat. No. 5,543,430 (8/96; filed 10/94), Moore et al., J. Biol. Chem. Vol. 251, No. 9, (5,76) pages 2788-2794, Sharma et al., J. Biol. Chem. Vol. 253, No. 18 (9/78) pages 6467-72 and Chem. Res. Tox. 1990 Vol. 3, pages 289-291."

Applicant respectfully requests clarification of the reasoning behind the combination of references cited in the rejection, as it is not understood what "if necessary" means. If there are alternatives in the combinations of references, or alternatives in the reasoning behind combining the references, Applicant requests that they be written out, so that there is opportunity for adequate response.

The teachings of WO 93/09806 and of Kaesemeyer (US 5,543,430) have been described above.

It is not understood what is meant by "the specification admission as to prior art on pages 37-39." No prior art is referred to that describes or suggests any attempted nitrosation, nitrosylation or nitrations of hemoglobin, or the use of any such form of hemoglobin in a method of therapy for a disorder resulting from platelet activation.

Moore *et al.* describe studies on nitrosylhemoglobin and nitrosylmyoglobin, performed in the absence of oxygen, in which dissociation of NO from the heme Fe of these molecules is followed spectrophotometrically. Moore *et al.* do not report or suggest any physiological effect of nitrosylhemoglobin or any other nitrosyl-heme containing NO donor.

Sharma *et al.* (*J. Biol Chem. 253*:6467-6472, 1978) describe studies on the dissociation of NO from the heme Fe of nitrosylhemoglobin, performed in the absence of oxygen. No studies of nitrosylhemoglobin and nitrosylmyoglobin are done under physiological conditions. Sharma *et al.* do not discuss any physiological effect of nitrosylhemoglobin or any other nitrosyl-heme containing NO donor. The high affinity of NO for heme (1,000 times that fo CO and 200,000

times that of oxygen, as reported in the fifth paragraph of page 272 of Greenburg and Kim, cited as reference AZ3) is incompatible with any NO donor effect.

Wade, R.S. and C.E. Castro (*Chem. Res. Tox. 3*:289-291, 1990) describe a reaction of nitrosoamines with heme to produce nitrosyl-heme products (Equation 1), a reaction of hemoglobin or myoglobin with nitric oxide to produce FeII-NO adducts (first paragraph), a reaction of metmyoglobin with nitric oxide to produce the heme-NO adduct (Equation 2), and the corresponding heme-NO adducts of the other heme proteins listed in Table 1 on page 289. Wade and Castro also describe (Equation 3) a reaction in which oxidized heme proteins, which are unable to carry oxygen, react with NO and a molecule providing a nucleophilic site (e.g., phenol), to produce a nucleophile-NO product (e.g., nitrosophenol), and the nitrosyl-heme protein. Nowhere does the reference describe a stable product of a reaction between a species of nitric oxide with a site on hemoglobin other than the heme Fe. The cited claims pertain to SNO-hemoglobin, an *S*-nitrosyl product, not an Fe-nitrosyl product. There is no suggestion in the reference that SNO-hemoglobin is possible to produce, and no suggestion of how to produce any product other than a heme-NO product in which NO is bound to the heme Fe.

The Examiner states:

> Additionally, the Stamler reference, besides generically describing the use of nitrosylated proteins (e.g. nitrosylhemoglobins), additionally suggests the use of *S*-nitrosylhemoglobin, for relaxing smooth muscle, inhibiting platelet aggregation, promoting vasodilation and for treating/preventing cardiovascular disorders, the Stamler reference fails to disclose other specific species nitrosylated hemoglobins (e.g. nitrosylhemoglobin, polynitrosated hemoglobin, and nitrosated methemoglobin).

WO 93/09806 discloses methods of thiol nitrosylation that were apparently successful for the production of some SNO-proteins. However, as presented by Applicants previously, nowhere is it disclosed in the prior art, including WO 93/09806, that these methods were successful in any synthesis of SNO-hemoglobin in particular. Hemoglobin is susceptible to dissociation of its subunits, denaturation, and oxidation of the heme Fe, and one of ordinary skill in the art would expect (as Applicants have confirmed experimentally as sworn to in the Declaration of Jonathan S. Stamler, M.D. Under 37 C.F.R. § 1.132 mailed to the Patent Office on September 2, 1999, and as stated to the Examiner personally in the interview of August 5, 1999) that hemoglobin would suffer these effects from treatment with acidified nitrite in 0.5 N HCl.

The Examiner further states that:

> . . . Stamler discloses different methods for thiol nitrosylation of proteins (as disclosed on page 30-31) which include:
> 1. reaction of nitrosylating agent (e.g. equimolar amounts of acidic $NaNO_2$ as nitrosating agent in a buffered saline at pH 7.4 for tPA):
> 2. exposure of the protein (e.g. tPA to NO gas in buffered saline) as well as Example 19 with respect to hemoglobin (e.g. See Example 19 on pages 58-59), which utilizes selection of a low molecular weight *S*-nitrosothiol (e.g. SNOAc) as nitrosating agent. Optimization, e.g., using "excess nitrosating agent" or higher pH values (e.g. pH 7.4) than that utilized in the specific thionitrosylated hemoglobin example (e.g. pH 6.9 Example 19) is within the skill of the art and is further suggested by Stamler since thionitrosylated proteins are known to be stable under physiological conditions (e.g. TBS, pH 7.4, room temperature; see page 31). See also other Examples which utilize physiological conditions in analogous steps. e.g., page 30, lines 20-27; page 33, lines 20-26).

It should be pointed out that the acidified nitrite procedure of nitrosylation of proteins (referred to as being on page 30, lines 20-27 and on page 33, lines 20-26 of WO 93/09806) was not carried out at pH 7.4, as the Examiner concludes, but at a far more acidic pH, in 0.5 N HCl, and the resulting solution was then brought to pH 7.4, after the nitrite had reacted, by the addition of NaOH and Tris buffered saline (TBS). WO 93/09806 does not suggest the use of any reaction that can be carried out at pH 7.4. Importantly, a reaction using $NaNO_2$ would not be successful in nitrosylation at pH 7.4; $NaNO_2$ will only work to nitrosylate an appropriate substrate under acidic pH conditions. $NaNO_2$ has the additional effect of oxidizing and destroying hemoglobin at all pHs.

The second method the Examiner states as disclosed in WO 93/09806 (exposure of the hemoglobin protein to NO gas in buffered saline, especially *excess* nitrosating agent) does not produce SNO-hemoglobin. That it is preferable to use a *low* ratio of NO:heme is demonstrated in Example 19, on page 87, and in Figure 17.

It is not understood how one can "optimize" reaction conditions, as the Examiner suggests is within the skill of one of ordinary skill in the art, if no successful reaction conditions have been demonstrated at all.

One of ordinary skill in the art would know from other references in the prior art that, whereas proteins such as tPA and albumin do not have heme groups, hemoglobin does, and that deoxyhemoglobin incubated with gaseous nitric oxide becomes nitrosylhemoglobin, in which NO is stably bound at the heme, and that oxyhemoglobin incubated with gaseous nitric oxide is oxidized to methemoglobin.

As stated previously, WO 93/09806 does not show the successful modification of hemoglobin to form SNO-hemoglobin. No spectrophotometric evidence is presented that would indicate the presence of SNO-hemoglobin. No results are presented from what is referred to in Example 19 as "standard methods for detection of *S*-nitrosothiols (Saville, *Analyst 83*:670-672, 1958)." In fact, the Saville assay was not routinely used for the detection of *S*-nitrosothiols, but was known only as a method for the quantitation of thiols, and had to be modified and adapted for effective use with SNO-hemoglobin. See the specification at page 78, line 14 to page 79, line 7. These modifications were not indicated in Example 19 of WO 93/09806 and would not be apparent to one of ordinary skill in the art. With no evidence that mixing SNOAc and hemoglobin produces any product, one of ordinary skill in the art would conclude that there is no reasonable expectation of success of any method given in WO 93/09806.

As discussed above, the Moore and Sharma references disclose studies on nitrosylhemoglobin and no other nitrosated forms of hemoglobin, but do not disclose any physiological function for nitrosylhemoglobin. The Moore *et al.* and Sharma *et al.* studies in no way imply that nitrosylhemoglobin can be a donor of nitric oxide or its biological equivalent, as nitrosylhemoglobin binds NO with an extremely high affinity. There can be no motivation drawn from the Moore or Sharma references to use nitrosylhemoglobin, either alone or in combination, for the treatment of any medical disorder, as nitrosylhemoglobin was not known at the time of the invention as an inhibitor of platelet aggregation, a vasodilator, an oxygen carrier, or for any physiological effect known to be possessed by NO or donors of NO.

Combining the references, one of ordinary skill in the art seeking a method to deliver NO or its biological equivalent to tissues in an animal or human, might turn to an *S*-nitrosoprotein or other *S*-nitrosothiol as described in WO 93/09806 or an inorganic nitrate as described in Kaesemeyer to be administered to a human or animal. *S*-nitrosohemoglobin was hypothesized to be an NO donor in WO 93/09806, but it cannot be synthesized according to any description in WO 93/09806 or elsewhere. Applicants later showed what WO 93/09806 did not and could not have anticipated, because *S*-nitrosohemoglobin was not actually made, that SNO-oxyhemoglobin does not act as an NO donor. The Wade and Castro paper and other references in the literature offer methods to produce proteins with NO bound to the heme Fe, to produce nitrosyl heme proteins such as nitrosylhemoglobin. However, one of ordinary skill in the art would know from

Moore, Sharma and other references in the literature that NO is extremely tightly bound to nitrosylhemoglobin, and that nitrosylhemoglobin is not a carrier of oxygen, and was not known to be a donor of NO or an intermediate in the formation of SNO-hemoglobin.

CONCLUSION

In his consideration of the patentability of the claims the Examiner has made and maintained rejections based on certain errors:

1) failing to give proper consideration and weight to the statements and scientific evidence presented in the Declaration of Jonathan S. Stamler, M.D. Under 37 C.F.R. § 1.132 and discussed in the interview conducted at the United States Patent and Trademark Office on August 5, 1999;
2) using the theory of inherency inappropriately in interpreting said Declaration;
3) requiring Applicants to meet a standard established by the Examiner only, and not based on the patent law, to rebut the Examiner's assertion that a reference sets forth an enabling description of an invention;
4) misinterpreting the prior art and drawing conclusions from it beyond what it teaches to one of ordinary skill in the art;
5) failing to properly consider the arguments of Applicants regarding the teachings of the cited prior art; and
6) failing to clearly set forth the reasoning in citing a combination of references in a rejection made under 35 U.S.C. § 103(a).

Because of the errors set forth above, the Examiner is respectfully requested to reconsider the rejections he has raised, and to withdraw them. If the Examiner feels that a telephone conference would expedite prosecution of this case, the Examiner is invited to call the undersigned attorney.

Respectfully submitted,

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